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Mycobacterium decipiens sp. nov., a new species closely related to the *Mycobacterium tuberculosis* complex

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Abstract

Two mycobacterial strains with close similarity to the *Mycobacterium tuberculosis* complex (MTBC) were isolated from cutaneous lesions of patients in the USA and Italy. At the phenotypic level, similarities to the MTBC included slow growth rate, rough morphotype of the unpigmented colonies and nearly identical high-performance liquid chromatography profiles of mycolic acids. In contrast to the MTBC, the strains were niacin- and nitrate-negative, and catalase-positive both at 68°C and in semi-quantitative tests. The clinical isolates were more closely related to *M. tuberculosis* than to any other known mycobacterium and scored positive with commercial DNA probes (Hologic AccuProbe *M. tuberculosis).* Both average nucleotide identity and genome-to-genome distance suggested the strains are different from the MTBC. Therefore, given the distinguishing phenotypic and genomic-scale differences, we submit that the strains belong to a new species we have named *Mycobacterium decipiens* with type strain TBL 1200985^T (=ATCC TSD-117^T=DSM 105360^T).

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Conflicts of interest

The authors declare that there are no conflicts of interest.

Keywords

Mycobacterium decipiens; *Mycobacterium tuberculosis* complex; average nucleotide identity; whole genome sequencing

Previously, a number of mycobacterial species have been reported to demonstrate high-level genotypic relatedness with the *Mycobacterium tuberculosis* complex (MTBC) and yield false positive results with commercial amplification methods [1] or with DNA probes specific for the MTBC [2, 3]. Here we describe a novel mycobacterium species closely related to the MTBC, but distinct from it.

The first strain, TBL 1200985^{T}, was isolated in 2012 in the USA from a 58-year-old female reporting swelling and pain at the right thumb and wrist for 8 months. Histology revealed granulomatous synovitis and a slowly growing nonpigmented mycobacterium grew by culture of two resected samples. The patient recovered after 18 months of therapy, including a standard anti-tuberculosis regimen which was subsequently adjusted to azithromycin and moxifloxacin [4].

A second strain, FI-16190, was isolated in 2016 from a 5- year-old girl in Italy presenting with abdominal swelling, pain and fever. A biopsy of the intra-abdominal lymph node revealed granulomatous lymphadenitis and yielded mycobacterium in culture.

The adult and child reported that their initial symptom onset was after returning from a vacation in a tropical area, the US Virgin Islands and the Republic of Maldives, respectively. In addition, upon returning from their trips, both the adult and child reported small wounds, at the hand and heel, respectively. An interphalangeal joint culture from the adult patient and an inguinal lymph node tissue culture from the child revealed acid-fast bacilli.

Non-pigmented rough colonies, morphologically compatible with the MTBC, developed in about 20 days at 25 and 37 °C. No pigmentation developed after light exposure. No growth was observed on standard media at 42 °C or on Mac-Conkey agar without crystal violet at 37 °C. Semi-quantitative and thermostable catalase tests were positive; the isolates also exhibited tellurite reduction. Unlike *M. tuberculosis,* the strains were negative for niacin accumulation, nitrate reduction, Tween 80 hydrolysis, urease activity and β-glucosidase activity [5].

The strains were positive with the MTBC-specific probes (Hologic AccuProbe) [6], while they were identified as non-specified *Mycobacterium* by GenoType CM (Hain Life- science) [7].

High-performance liquid chromatography profiles of cell-wall mycolic acids [8] of the two strains were obtained using the Sherlock Mycobacteria Identification System (SMIS; midi) and produced a pattern of five major peaks eluting between 7 and 8min (Fig. 1). This profile closely resembles that of *M. tuberculosis* (Sherlock software similarity index 0.749).

The determination of minimum inhibitory concentrations (MICs) of antimicrobials potentially active against slow-growing mycobacteria [9] was performed using commercial

plates (Sensititre slomyco, Thermo Fisher Scientific). Both strains showed susceptibility to seven agents: amikacin, clarithromycin, doxycycline (susceptible/intermediate), line-zolid, moxifloxacin, rifabutin and trimethoprim-sulfameth-oxazole, with resistance to rifampicin. Differences in susceptibility to ethambutol and ciprofloxacin were observed between the two strains (Table 1).

The whole genome sequences of both clinical isolates were produced using the Illumina platform and Nextera reagents (Illumina) according to the manufacturer's protocol. Reads were quality trimmed with TrimGalore and assembled using SPAdes version 3.9.1 software [10]. The genomes of TBL 1200985^T and FI-16190 exhibited similar features (Table 2).

DNA gene sequences of the entire 16S rRNA gene and of the hypervariable regions of the *hsp65* (401 bp) [11] and *rpoB* (720 bp) genes [12] were extracted from the genomic sequences of the two strains. TBL 1200985^T and FI-16190 had identical 16S rRNA and *hsp65* sequences while the *rpoB* sequences differed by only 1 bp. Their 16S sequences were 99.4 % similar to *M. tuberculosis,* and consequently with all other members of the MTBC, with only seven basepair mismatches. For the *hsp65* gene, a large number of species, including MTBC members, were characterized by similarity around 94–95 %, with *Mycobacterium intracellulare* being the closest (95.3 %). For *rpoB,* the clinical isolates diverged from every known species, with *M. tuberculosis* exhibiting the highest resemblance (89.5 and 89.7 % in the two strains).

In addition, the *hsp65* gene hypervariable region allowed us to infer the PRA (PCR restriction analysis) pattern of the isolates. The enzyme *BstE*II produced two restriction fragments of 310 and 116 bp, while *Hae*III produced three fragments of 127, 112 and 69 bp. *Mycobacterium kumamo-tonense* and *Mycobacterium gordonae* were the species presenting the closest resemblance with this pattern whilst the MTBC patterns were clearly different.

Due to high 16S rRNA gene similarity (99.4 %) between the clinical isolates and the MTBC, the calculation of the average nucleotide identity (ANI) [13] was needed [14]. Both TBL 1200985^T and FI-16190 scored lower than the boundary of species (95–96%) in comparison to different members of the MTBC (Table 3 and Fig. S1, available in the online version of this article). As noted in Table 3, one isolate of each of seven species of the MTBC were used for comparison. These species show >99 % similarity to each other, which suggests they represent a single species, as discussed in a recent paper [15]. An analogous result was achieved by calculating the genome-to-genome distance (GGD) [16] (http://ggdc.dsmz.de/), the *in silico* equivalent of DNA-DNA hybridization (Table 3).

To perform phylogenetic analysis, the sequences of closely related mycobacterium species were retrieved from Gen-Bank, aligned with CLUSTAL_W [17] and trimmed to start and end at the same nucleotide position. The neighbour-joining method using the Tamura-Nei distance model [18, 19] with 1000 bootstrap replicates was used to reconstruct the trees based on 16S rRNA, *hsp65* and *rpoB* gene sequences individually and concatenated. *Mycobacterium abscessus* was chosen as an outgroup. Different trees placed TBL 1200985^T and FI-16190 within the same clade as *M. tuberculosis* (Figs 2 and 3, S2 and S3).

DESCRIPTION OF MYCOBACTERIUM DECIPIENS SP. NOV.

Mycobacterium decipiens (de.ci'pi.ens L. part. adj. *decipiens,* deceiver, characterized by deceptive features leading to mis-identification as *M. tuberculosis).*

Non-motile, non-spore-forming and acid fast. Grows on solid media in approximately 15–20 days at temperatures ranging from 25 to 37 °C, produces rough colonies, and is non-pigmented regardless of exposure to light or dark conditions. The species differs from *M. tuberculosis* in its inability to accumulate niacin or to reduce nitrates, and in its production of thermostable catalase at high (>45 mm) levels. The commercial AccuProbe misidentified *M. decipiens* as a member of the MTBC. In the 16S rRNA and *rpoB* genes, the species is most closely related to the MTBC. The strains were largely susceptible to most antimicrobials, with both being resistant to only rifampicin. The ANI between respective genomes (confirmed by GGD) is supportive of the status of species as independent from the MTBC. The genome sizes and number of coding DNA sequences of the clinical isolates was 5 228 890 bp and 4878 genes (TBL 1200985^T), and 5 432 050 bp containing 4676 genes (FI-16190).

The type strain is TBL 1200985^T (=ATCC TSD-117^T=DSM 105360^T).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA, hsp65 and rpoB gene sequences of strain TBL 1200985 are KF683289, KJ371035 and KJ371034; the rpoB gene sequences of strain FI-16190 is KY657270. The accession number of the shotgun gene sequence of strain TBL 1 200985 is NCXP00000000.

Abbreviations:

ANI	Average nucleotide identity
GGD	Genome-to-genome distance
HMMIS	High molecular mass internal standard
LMMIS	Low molecular mass internal standard
MIC	Minimum inhibitory concentration
MTBC	Mycobacterium tuberculosis complex

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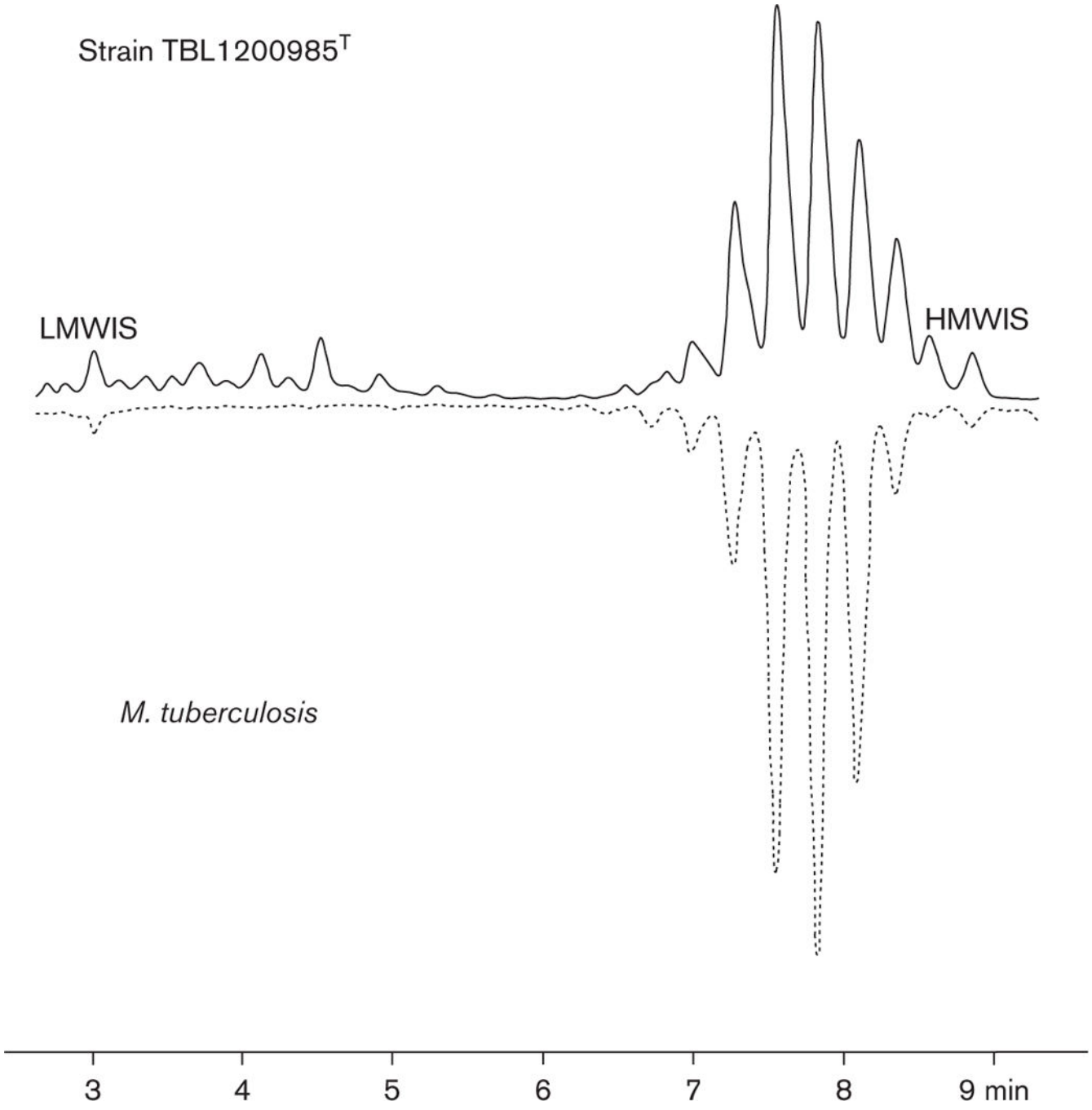

Fig. 1.

Representative mycolic acid patterns of strain TBL 1 200985^T and *M. tuberculosis.* LMMIS, low molecular mass internal standard; HMMIS, high molecular mass internal standard.

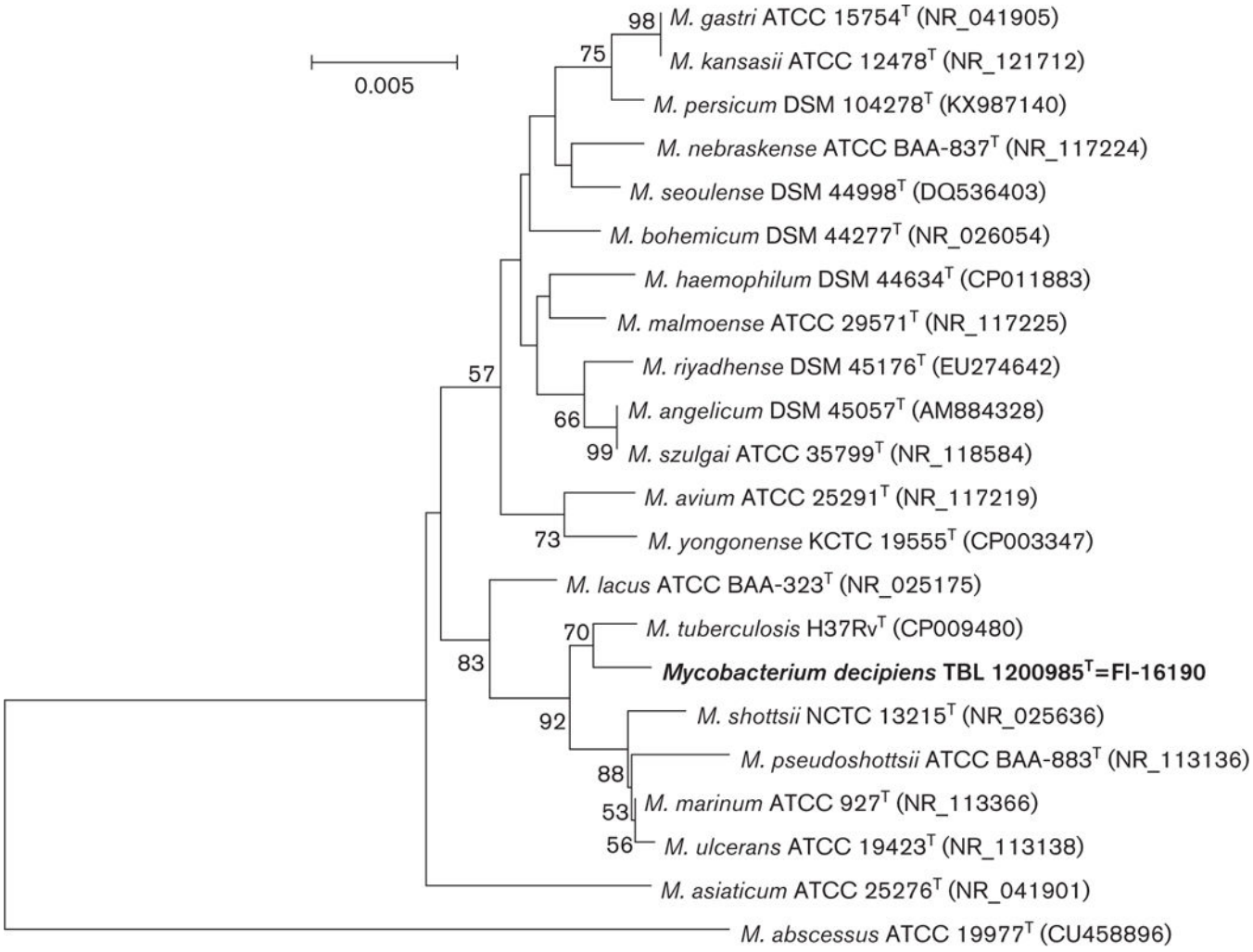

Fig. 2.

Phylogenetic tree based on 16S rRNA sequences of representative species of the genus *Mycobacterium*, reconstructed using the neighbour-joining method bootstrapped 1000 times. Bootstrap values >50 are given at nodes. Bar, 0.005 substitutions per nucleo-tide position.

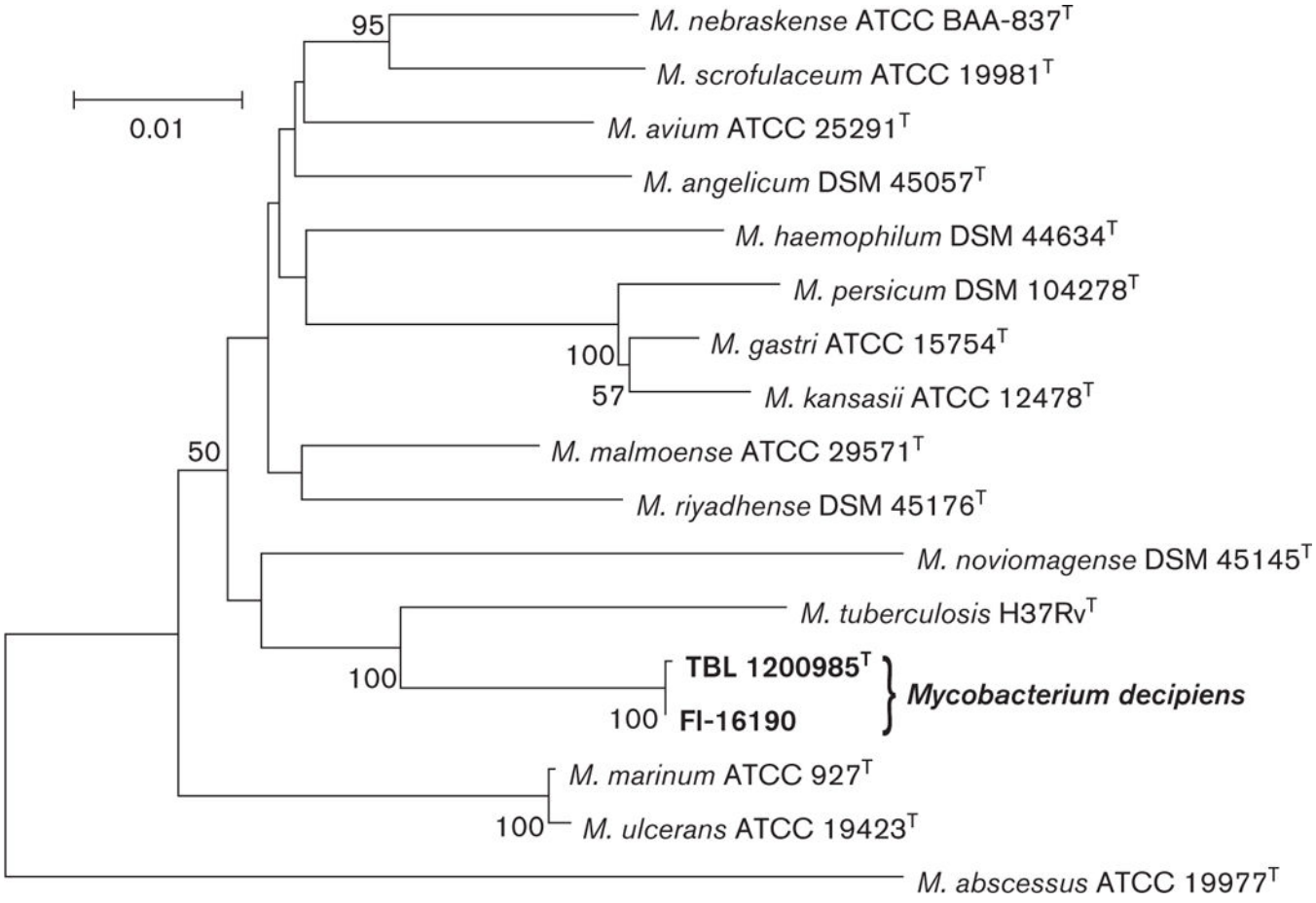

Fig. 3.

Phylogenetic tree based on concatenated sequences of 16S rRNA, *hsp65* and *rpoB* gene sequences of representative species of the genus *Mycobacterium*, reconstructed using the neighbour-joining method bootstrapped 1000 times. Bootstrap values >50 are given at nodes. Bar, 0.01 substitutions per nucleotide position.

Table 1.

Minimum inhibitory concentrations (MICs) of the clinical isolates S, susceptible; I, intermediate; R, resistant.

Antimicrobial tested	TBL 1200985^T MIC (μg ml $^{-1}$) (Interpretation)	FI-16190 MIC (μg ml $^{-1}$) (Interpretation)
Amikacin	≤1 (S)	4 (S)
Ciprofloxacin	4 (R)	1 (S)
Clarithromycin	4 (S)	0.25 (S)
Doxycycline	0.5 (S)	2 (I)
Ethambutol	4 (I)	8 (R)
Linezolid	≤1 (S)	≤1 (S)
Moxifloxacin	0.5 (S)	≤0.12 (S)
Rifabutin	≤0.25 (S)	≤0.25 (S)
Rifampicin	2 (R)	2 (R)
Trimethoprim/sulfamethoxazole	0.25/4.75 (S)	0.5/9.5 (S)

Table 2.

Salient genomic features of the *Mycobacterium decipiens* clinical isolates CDS, coding DNA sequences.

Strain	Mean coverage	Number of contigs	G+C content (%)	Genome size (bp)	Number of CDS
TBL 1200985^{T}	44.9×	142	65.4	5 228 000	4878
FI-16190	44.1×	176	65.5	5 432 000	4676

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Table 3.

ANI and (GGD) % scores between members of *M. tuberculosis* complex and *Mycobacterium decipiens* clinical isolates*

	Accession number	TBL 1200985^T		*M. tuberculosis*		*M. africanum*		*M. bovis* BCG		*M. bovis*		*'M. canettii'*	*M. caprae*	*M. microti*
M. tuberculosis H37Rv	NC_000962	86.02 (30.20)												
M. africanum strain 25	CP010334	86.07	(30.20)	99.81	(96.70)									
M. bovis BCG ATCC 35743	NZ_CP003494	86.04 (30.10)		99.77 (96.70)		99.77 (95.70)								
M. bovis ATCC BAA935	NZ_CP009449	86.10	(30.20)	99.79	(96.60)	99.78	(96.40)	99.75 (96.60)						
'M. canettii' CIPT 140010059	NC_015848	86.03	(30.20)	99.25	(91.20)	99.24	(91.40)	99.16	(89.80)	99.20	(90.50)			
M. caprae strain Allgeau	NZ_CP016401	86.08	(30.20)	99.86	(97.40)	99.81	(97.30)	99.82	(96.90)	99.81	(97.10)	99.27 (91.50)		
M. microti strain 12	CP010333	86.05	(30.10)	99.85	(97.10)	99.82	(98.00)	99.75	(96.10)	99.78	(93.50)	99.27 (91.50)	99.85 (97.40)	
FI-16190	–	99.63	(97.30)	86.02	(30.10)	85.92	(30.10)	85.95	(30.10)	85.94	(30.10)	86.01 (30.10)	85.96 (30.20)	85.87 (30.10)

*ANI scores <95 % and GGD scores <70 % are indicative of belonging to different species; ANI scores >96 % and GGD scores >70 % are indicative of belonging to the same species.